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IN-VITRO EVALUATION OF TRICHODERMA ISOLATES AGAINST MAJOR SOIL BORNE PATHOGENS IN GROUNDNUT (*ARACHIS HYPOGAEA* L)

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ABSTRACT

Nine Trichoderma spp. Isolates were collected from different cropping systems (Groundnut, Red gram, and Tomato) in Chittoor district, and evaluated against three major soil borne pathogens of groundnut such as Aspergillus Niger, Sclerotium rolfsii and Macrophomina phaseolina. Against A. Niger, GRT-3 isolate showed maximum percent of inhibition (65.87%) followed by TRT-2 (65.50%). Case of S. rolfsii, TRT-1 isolate showed maximum per cent of inhibition (68.75%) followed by GRT-1 (68.00%). The highest inhibition zone was recorded in GRT-3 (0.50cm) with 57.50 per cent inhibition followed by TRT-2 (0.43cm). Against M. phaseolina, TRT-2 isolate showed maximum per cent of inhibition (70.50%) followed by TRT-1 (70.00%), but isolate GRT-3 overgrew pathogen (.3.05cm) with sporulation, as in case of RRT-1 and RRT-2 overgrew pathogen (3.50cm) without sporulation.

KEYWORDS: Trichoderma Spp., Aspergillus Niger, Sclerotium Rolfsii and Macrophomina Phaseolina & Dual Culture

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Original Article

INTRODUCTION

Out of nine oilseed crops grown in India, groundnut accounts for 35% of the total area cropped under oilseed and 40% of the total oilseed production. Though, India is the largest producer of groundnut, its average productivity levels are very low as compared to the USA and China. An important factor contributing to low yield are diseases. Among all the diseases, soil borne diseases such as collar rot, stem rot and root rot caused by Aspergillus Niger, Sclerotium rolfsii and Macrophomina phaseolina, respectively are serious problems. Ghewande et al. (2002) reported the losses in terms of mortality of plants, due to collar rot were in the range of 28 to 50 per cent. Stem rot disease causes severe damage during any stage of crop growth, and yield losses over 25% have been reported by Mayee and Datar (1988). Moreover, most of the varieties are susceptible to this disease. Many seed dressing fungicides are reported to be effective against all soil borne pathogens of groundnut (Gangopadhyay et al., 1996; Karthikeyan, 1996), but the pathogens may develop resistance to fungicides. Besides, these the chemicals causes damage to agro-ecosystem in the soil.

Trichoderma species are asexual, soil-inhabiting filamentous fungi and have the ability of antagonizing a series of plant pathogenic fungi (Papavizas, 1985). Proposed mechanisms of antagonism include mycoparasitism by the action of cell-wall degrading enzymes, antibiosis by the production of antibiotics, competition for space and nutrients through rhizosphere competence, facilitation of seed germination and growth of the plants via releasing important minerals and trace elements from the soil and induction of the defense responses in plants (Herrera and Chet, 2003). The advantage of using *Trichoderma* in managing soil borne plant pathogens is ecofriendly, effective, ease of

mass culturing with less cost of production and growth promoting effect. Taking into consideration the above facts, the present investigation has been formulated to know the antagonism of rhizospheric Trichoderma spp. against soil borne pathogens *S. rolfsii, A. niger and M.phaseolina*

MATERIAL AND METHODS

Isolation and Maintenance of Soil Borne Pathogens and *Trichoderma* Spp

Groundnut plants showing seedling disease symptoms were collected from the fields of Chittoor district. Groundnut plants showing typical disease symptoms were selected for isolation of test pathogens *Aspergillus Niger*, *Sclerotium rolfsii* and *Macrophomina phaseolina*. *Trichoderma* spp. These were isolated using *Trichoderma* Selective Medium (TSM) from different cropping systems such as groundnut, radiogram and tomato fields in Chittoor district, Andhra Pradesh, by following serial dilution technique (Johnson and Curl, 1977). Pure cultures of these pathogens and antagonist were maintained on Potato dextrose agar (PDA) slants and stored in 4° C for further studies.

In Vitro Antagonism between Bio-Agent *Trichoderma* and Pathogen

Individual *Trichoderma* isolate was dual cultured with test pathogen. Twenty ml of melted and cooled PDA medium was poured into Petri plates and allowed to solidify. Five mm cutter disc of *Trichoderma* was placed 1cm away at one end of Petriplate. A 5 mm test pathogen culture disc was placed 1cm away at the opposite end (With a gap of 7 cm between the two culture discs) (Morton and Straube, 1955). Plates manufactured with either of the test fungi served as a check. Three replications were maintained for each treatment. The percent inhibition of radial growth of the test pathogen was calculated by using the following formula.

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent reduction in growth of test pathogen, C = Radial growth (cm) in control, T = Radial growth (cm) in treatments

RESULTS AND DISCUSSIONS

All the three pathogens were isolated using a potato dextrose agar medium (Figure 1). A total of nine *Trichoderma* isolates were obtained from twenty seven rhizosphere samples (Figure 2). The antagonistic *Trichoderma* spp were identified based on mythological keys described by Barnett *et al.* (1972).

In order to know the antagonistic potential of *Trichoderma* spp. isolates against all the pathogens i.e. all nine isolates were screened against the test pathogens by using the dual culture technique. *Trichoderma* isolates showed significant reduction in mycelial growth of test pathogens. The interactions of *Trichoderma* isolate with *A. Niger* was recorded as isolate GRT-3 showed maximum percentage of inhibition (65.8%), followed by TRT-2 (65.50%). The inhibition percentage of other isolates in descending order is as GRT-2 (64.62%), TRT-1 (63.37%), RRT-2 (62.12%), GRT-4 (61.25%), GRT-1 (60.87%), GRT-5 (59.62%), RRT-1 (58.75%) (Table 1). The highest zone of inhibition (0.4 cm) was observed by isolating GRT-4 and RRT-1, followed by GRT-5 and TRT-1 (0.3cm) (Table 2). Least zone of inhibition was observed in GRT-1 (0.1cm). A zone of inhibition was not observed in isolated GRT-3 and RRT-2. (Table 2, Figure 3).

Gajera *et al*. (2011) studied the antagonistic effect of 12 isolates of three *Trichoderma* spp (*T. virens, T. viride, T. harzianum*) against the collar rot causing fungus *A. Niger*. *T. viride* 60 showed maximum growth inhibition (86.2%) followed by *T. harzianum* 2 (80.4%).

Against *S. Roofs*, TRT-1 isolate showed the maximum percentage of inhibition (68.75%) followed by GRT-1 (68.00%). The inhibition percentage of other isolates in descending order is as GRT-2 (65.00%), RRT-2 (62.50%), TRT-2 (60.87%), GRT-5 (60.00%), GRT-3 (57.50%), GRT-4 (56.25%), RRT-1 (53.75%) (Table 1).

Table 1: In Vitro Screening of Trichoderma Isolates against *A.* Niger, *S.* Rolfsii, *M.* Phaseolina Pathogens by Dual Culture Technique at Sixth Day

Isolate	Diameter Growth of *A. Niger* (cm)	Per Cent Inhibition Over Control	Diameter Growth of *S. Rolfsii* (cm)	Per cent Inhibition Over Control	Diameter Growth of *M.phaseolina* (cm)	Per cent Inhibition Over Control
GRT-1	3.13	60.87	2.56	68.00	2.83	64.60
GRT-2	2.83	64.62	2.80	65.00	2.93	63.33
GRT-3	2.73	65.87	3.40	57.50	2.50	68.75
GRT-4	3.10	61.25	3.50	56.25	2.60	67.50
GRT-5	3.23	59.62	3.20	60.00	2.70	66.25
RRT-1	3.30	58.75	3.70	53.75	2.60	67.50
RRT-2	3.03	62.12	3.00	62.50	2.53	68.30
TRT-1	2.93	63.37	2.50	68.75	2.40	70.00
TRT-2	2.76	65.50	3.13	60.87	2.36	70.50
Control	8.00	0.00	8.00	0.00	8.00	0.00
CD	0.113		0.193		0.13	
CV	1.868		3.144		2.529	

GRT = Groundnut Rhizosphere *Trichoderma*, RRT = Redgram Rhizosphere *Trichoderma*, TRT = Tomato Rhizosphere *Trichoderma*

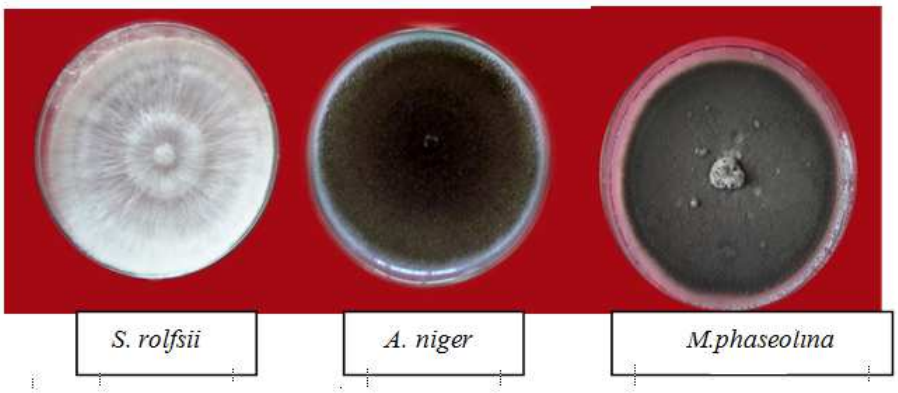

Figure 1: Pure Cultures of Soil Borne Pathogens

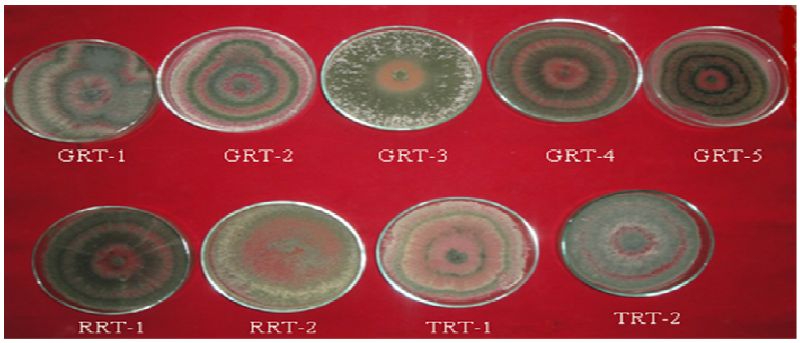

Figure 2: Pure Cultures of Trichoderma Isolates from Rhizosphere Region of Groundnut Red gram and Tomato

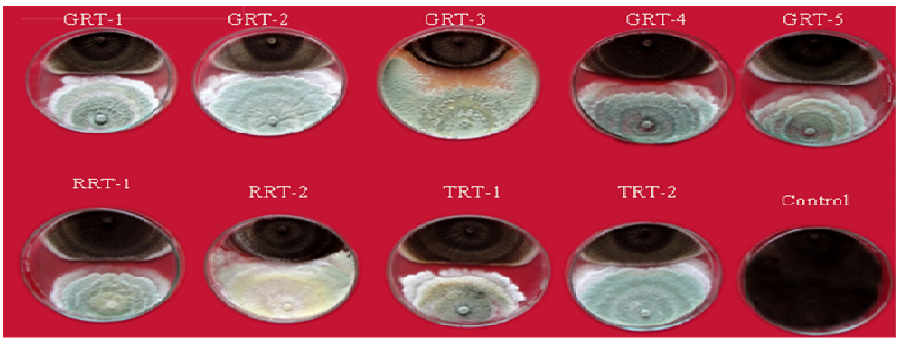

Figure 3: In Vitro Efficacy of Trichoderma Isolates on Mycelial Growth of Aspergillus Niger in Dual Culture Technique

Table 2: Radial Growth of *Trichoderma* Isolates and *A. Niger* in Dual Culture Plates at Fifth Day

S. No	*Trichoderma* Isolate	*A. Niger* Growth (cm)	*Trichoderma* Growth (cm)	Over Growth of *Trichoderma* (cm)	Over growth of *A. Niger* (cm)	Zone of Inhibition (cm)
1	GRT-1	3.13	3.77	-	-	0.10
2	GRT-2	2.83	3.97	-	-	0.20
3	GRT-3	2.73	4.27	-	-	-
4	GRT-4	3.10	3.50	-	-	0.40
5	GRT-5	3.23	3.47	-	-	0.30
6	RRT-1	3.30	3.30	-	-	0.40
7	RRT-2	3.03	3.97	-	-	-
8	TRT-1	2.93	3.77	-	-	0.30
9	TRT-2	2.76	4.04	-	-	0.20
10	Control	8.00	8.00	-	-	-

Indicates no over growth/ zone of inhibition

On fourth day, an interaction between *Trichoderma* isolates and *S. rolfsii*, overgrowth of *Trichoderma* on pathogen and the overgrowth of pathogen on *Trichoderma* were recorded. Among all the nine isolates tested, the highest inhibition zone was recorded in GRT-3 (0.50cm), followed by TRT-2 (0.43cm). Least zone of inhibition was recorded in GRT-1 (0.10cm). No inhibition zone was reported in GRT-4, RRT-1 and RRT-2. Isolate RRT-1 showed highest over growth of *Trichoderma* (0.53 cm) on pathogen, followed by GRT-4 and TRT-1 observed 0.40 cm overgrowth on pathogen. However, by fifth day *S. rolfsii* started overgrowth on GRT-2 (0.40 cm). This indicated that *S. rolfsii* antibiotics were potential and GRT-2 succumbed to them, facilitating overgrowth of *S. rolfsii* (Table 3, Figure 4).

Table 3: Radial Growth of *Trichoderma* Spp. Isolates and *S. Rolfsii* in Dual Culture Plates at Fourth Day

S. No.	*Trichoderma* Isolate	*S. Rolfsii* Growth (Cm)	*Trichoderma* Growth (Cm)	Over Growth of *Trichoderma* (Cm)	Over Growth of *S.Rolfsii* (Cm)	Zone of Inhibition (Cm)
1	GRT-1	2.56	4.73	0.30	-	0.10
2	GRT-2	2.80	4.20	-	0.40	0.40
3	GRT-3	3.40	3.10	-	-	0.50
4	GRT-4	3.50	3.90	0.40	-	-
5	GRT-5	3.20	3.40	-	-	0.40
6	RRT-1	3.70	3.83	0.53	-	-
7	RRT-2	3.00	4.00	-	-	-
8	TRT-1	2.50	4.90	0.40	-	0.20
9	TRT-2	3.13	3.50		-	0.43
10	Control	8.00	8.00	-	-	-

Indicates no over growth/ zone of inhibition

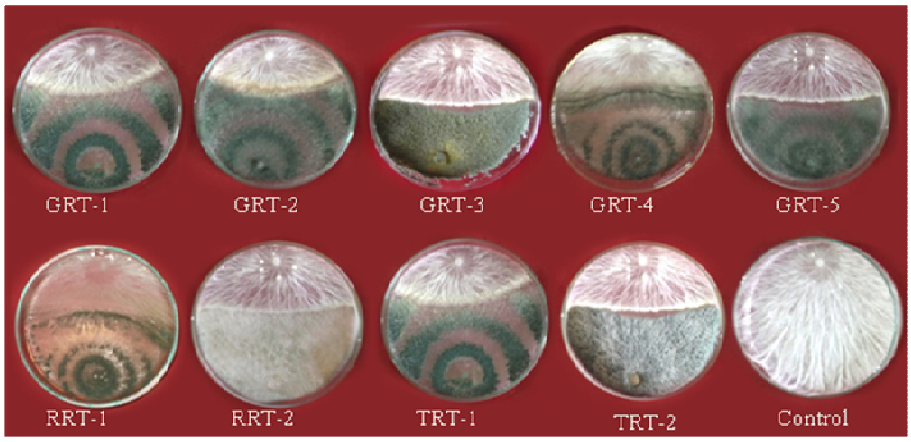

Figure 4: In Vitro Efficacy of Trichoderma Isolates on Mycelia Growth of *S.* Rolfsii in Dual Culture Technique

These results were in agreement with Sonali and Gupta (2004), who reported maximum reduction (72.22%) of mycelial growth of *S. rolfsii* in dual cultures by *Trichoderma viride,* among mycoflora isolated.

Against *Macrophomina phaseolina*, TRT-2 isolate showed the maximum percentage of inhibition (70.50%) followed by TRT-1 (70.00%) and GRT-3 (68.75%). The inhibition percentage of other isolates in descending order as RRT-2 (68.30%), GRT-4 (67. 50%), RRT-1 (67. 50%), GRT-5 (66.25%), GRT-1 (64.60%), GRT-2 (63.33) (Table 1). On the fourth day, interaction between *Trichoderma* isolates and *M. Gasoline* resulted in either overgrowth of *Trichoderma* on the pathogen or the overgrowth of pathogens on *Trichoderma* (Table 4). None of the isolates showed zone of inhibition against pathogens. Isolate GRT-3 overgrew pathogen (3.50cm) with sporulation, as in case of RRT-1 and RRT-2 overgrew pathogen (3.50cm) without sporulation (Figure 5).

Lokesha and Benagi (2007) reported that efficiency of *Trichoderma* spp. was more than 78.22% in dual culture method, when they worked on biological control of *M. phaseolina.*

Figure 5: In Vitro Efficacy of Trichoderma Isolates on Mycelia Growth of Macrophomina Phaseolina in Dual Culture Technique

Table 4: Radial Growth of Trichoderma Isolates and Macrophomina Phaseolina in Dual Culture Plates at Fourth Day

S.No.	Trichoderma Isolate	Macrophomina Growth(cm)	*Trichoderma* Growth(cm)	Over Growth of Trichoderma(cm)	Over Growth of *Macrophomina*(cm)	Zone of Inhibition(cm)
1	GRT-1	2.83	4.16	-	-	-
2	GRT-2	2.93	4.13	-	-	-
3	GRT-3	2.50	8.00	3.50	-	-
4	GRT-4	2.60	7.00	2.60	-	-
5	GRT-5	2.70	7.20	2.90	-	-
6	RRT-1	2.60	7.90	3.50	-	-
7	RRT-2	2.53	8.00	3.50	-	-
8	TRT-1	2.40	7.20	0.20	-	-
9	TRT-2	2.36	4.83	0.20	-	-
10	Control	8.00	8.00	-	-	-

Indicates no over growth/ no inhibition

CONCLUSIONS

In dual culture, among the nine *Trichoderma* spp. isolates tested against *A. Niger*, GRT-3 isolate showed the maximum percentage of inhibition (65.87%), followed by TRT-2 with (65.50%). A zone of inhibition was not observed in isolated GRT-3 and RRT-2. In case of S. *rolfsii*, TRT-1 isolate showed maximum percentage of inhibition (68.75%), followed by GRT-1 (68.00%). The highest inhibition zone was recorded in GRT-3 (0.50cm), with 57.50% percentage inhibition followed by TRT-2 (0.43cm). Against *M. phaseolina*, TRT-2 isolate showed maximum percentage of inhibition (70.50%), followed by TRT-1 (70.00%), but isolate GRT-3 overgrew pathogen (3.50cm) with sporulation, as in case of RRT-1 and RRT-2 overgrew pathogen (3.50cm) without sporulation. Hence, fungal antagonist (*Trichoderma* spp.) found effective against above tested soil borne microorganisms.

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